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chains, and refining a reasonable model. CaBLAM uses carbonyl oxygen placement to diagnose commonly occurring patterns of correctable backbone errors, then uses contours derived from a high-quality dataset in a novel parameter space of overlapping Cα pseudo-dihedrals to identify the secondary structures disguised by those errors.
In addition to continuous secondary structures like alpha helix and beta sheet, CaBLAM can identify non-continuous secondary structures such as helix caps, tight turns, and beta bulges, even in low-resolution models. The power to distinguish between modeling errors and these real irregularities will provide refinement with a more realistic, more detailed protein backbone from which to produce a reliable structural model.

105-Plat
Automated Circular Dichroism Spectroscopy for Medium-Throughput Quantification of Protein Conformation
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Circular dichroism (CD) spectroscopy is a powerful method for monitoring conformational changes of biomolecules. For proteins, it is highly sensitive to changes in secondary structure, which, in turn, are influenced by amino acid composition, posttranslational modifications, solution conditions (e.g., temperature, pH, salts, detergents, denaturants, excipients, etc.), and ligand binding. The CD signal is directly linked to protein structure, the analyte is in solution and label-free, the technique requires low sample amounts, and data analysis is straightforward. However, CD spectroscopy has remained a low-throughput method because it imposes high requirements on the optical quality of sample cells and thus cannot be performed in microplate-reader format. Here, we introduce an automated CD spectrometer that is equipped with a flow-through cell and coupled to a 3-axis robotic liquid handler. This enables completely unattended CD measurements on up to 384 samples, including sample transfer from 96-well plates into the flow-through cell, data acquisition, and cell cleaning. We demonstrate that the accuracy, precision, and data quality of the automated CD spectrometer are as good as those of a conventional, manually operated instrument and exemplify how the advantages offered by automated CD spectroscopy can be exploited in protein-unfolding experiments using chemical denaturants.

Platform: Membrane Active Peptides & Toxins

106-Plat
Probing the Membrane Interaction of Antimicrobial Agents In Vivo with Intact Bacteria by 2H NMR
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The cell membrane is an important biological interface often targeted by bioactive molecules such as antimicrobial peptides (AMPs) which can disrupt the bacterial lipid barrier. Identification and study of AMPs is a dynamic research field motivated by the emergence of antibiotic-resistant pathogens. To develop new efficient therapies, the action mechanism of membrane-disrupting antimicrobial agents should thus be studied. 2H solid-state NMR is a useful method to monitor changes in organization and dynamics of phospholipids in bilayers. Usually carried out with model systems, the complex composition of bacterial membranes - especially the presence of lipopolysaccharides (LPS) - encourages such studies on intact organisms. We have therefore developed a protocol to 2H-label phospholipids in Escherichia coli membranes without mutating. About 75% of the phospholipids had deuterated acyl chains. We have thus investigated in vivo the interaction mechanism of antimicrobial agents, i.e., the antibiotic polymyxin B (PxB), the detergent cetyltrimethylammonium chloride (CTAC) and fullerenol nanoparticles. 2H NMR spectra and spectral moment analysis support the insertion of the PxB's lipid tail in the bacterial membrane. Our results also suggest that membrane rigidification could play a role in the biocide activity of the detergent. The use of intact bacteria seems important in cases where the antibiotic action relies on an interaction with LPS. This is shown with fullerenol nanoparticles for which a different effect is seen on intact cells as compared to model DPPC/DPPG membranes, as will be detailed. This is further demonstrated with the blue pigment marennine produced by marine microalgae which would also interact with LPS. A different perturbation of the bacterial membranes by the intra- and extracellular forms of this pigment was also evidenced, thus shedding light on the action mechanism of this potential natural antibiotic.

107-Plat
Molecular Modeling of the Attachment of the Dengue II Envelope Protein into Host Endosomal Membranes
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Dengue virus is a world health threat responsible for 50-100 million infections per year with a 0.077% mortality rate. Its outer envelope is composed of an icosahedral shell of 180 copies of a class II fusion peptide, responsible for cell recognition and for escape from the host endosome via pH-induced rearrangement into a trimer. The lipid makeup of the endosomal membrane can block viral association and release, with homologous members of the flavivirus family requiring different key factors. We report results of the first molecular dynamics calculations on the complete envelope protein trimer at the membrane interface (21-aa segments were studied in Biochem. 49:287, 2010). The insertion energy profile and pattern of host membrane deformation upon viral association indicate that the insertion process is not highly energetically favorable. Instead, it is hypothesized that membrane association and viral fusion are mediated by binding of individual lipids to the tip of the viral envelope protein trimer. Our results explain the fusogenic activity of the isolated fusion peptide fragment, and suggest an alternative mechanism for the experimentally observed specificity to membrane composition.

108-Plat
Bacterial Biofilm Formation Induces Strong Shifts in Lipid Composition Resulting in Increased Resistance Towards Antimicrobial Peptide Activity
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Bacteria that interact with surfaces under hydrated conditions can form aggregated structures known as biofilms. Biofilms are characterized by having increased resistance to a variety of antibacterial agents. This resistance is responsible for the generation of persistent chronic infections, and represents a serious threat to human health. Several antimicrobial agents, including hydrolytic enzymes such as PLA2-IIA and antimicrobial peptides (AMPs) such as Magainin-2, act by disrupting bilayer membrane integrity. Since these antimicrobial agents require physical disruption of the bilayer membrane, their activity is likely to be sensitive to lipid packing.. In this work we show, by measuring generalized polarization of Laurdan incorporated into lipid extracts, drastic changes in the level of lipid packing in Staphylococcus aureus during biofilm formation. When analyzing lipid composition we find a significant reduction in the level of branched lipids in the biofilm membranes. A strong reduction in the level of carotenoids is also observed during biofilm formation. Additionally, we present evidence that this shift in the melting temperature modulates resistance towards magainin-2 at 37C where bacterial lipids are in the liquid-crystalline phase. These results point to a mechanism by which bacterial membranes can generate resistance towards membrane active antibacterial agents through the modulation of lipid composition during biofilm formation.

109-Plat
Antimicrobial Peptide Activity in a Competitive Membrane Lipid Environment
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Mastering the structure-activity relationship and specificity of antimicrobial peptides (AMP) against bacterial lipid membranes is required for the therapeutic development of membrane-active peptides. Correlation of physiological observations with in vitro studies, including high resolution structural work, can provide the required understanding of the mechanism by which AMP kill bacteria. For instance, maculatin 1.1 is an antimicrobial peptide that serves as part of the innate immune defences of an Australian tree frog, and has shown promising activity against methicillin-resistant Staphylococcus aureus but which also has appreciable haemolytic activity. Against that common assumption that lipid composition is often assumed to be the regulative mechanism,